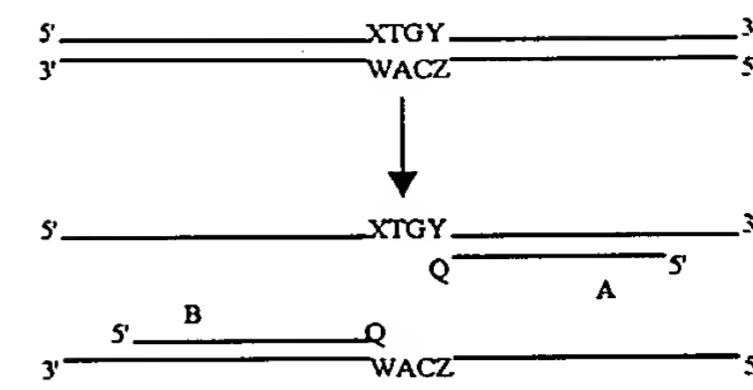
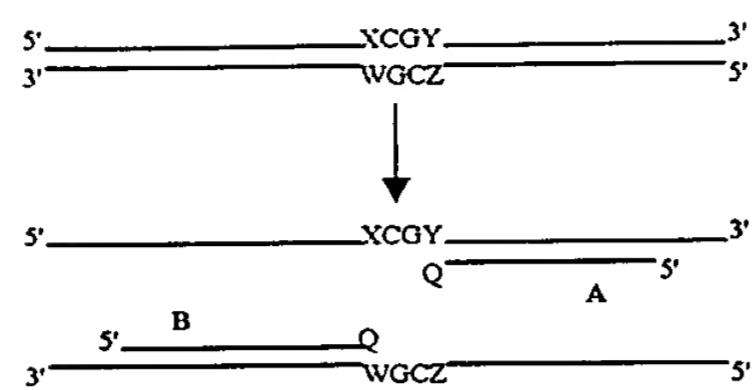


STEPS IN
PCR/RE/LDR

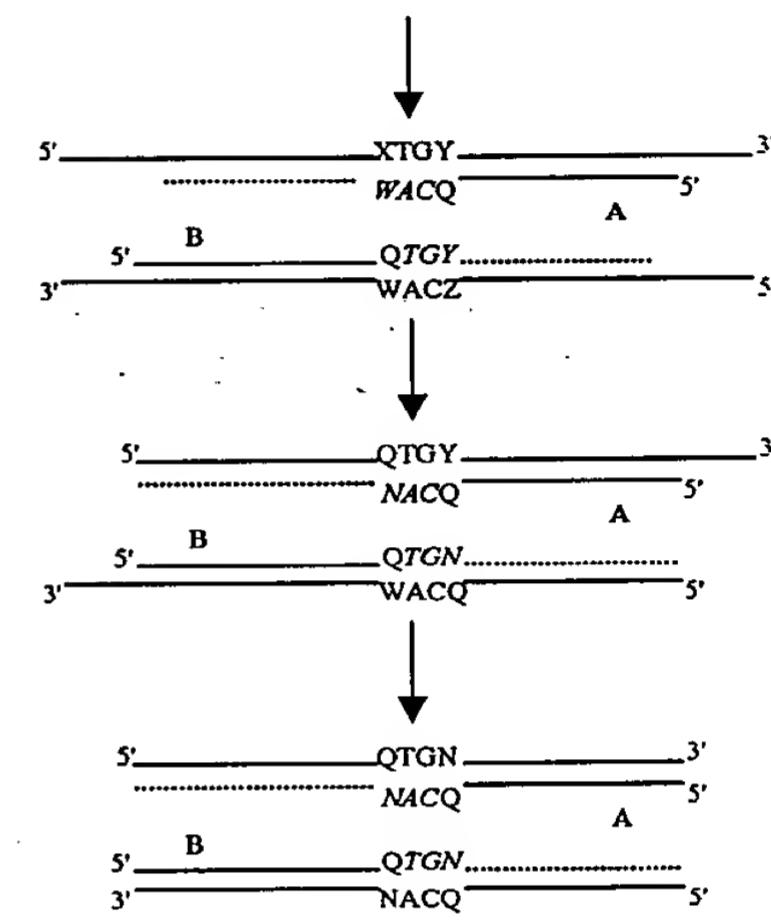
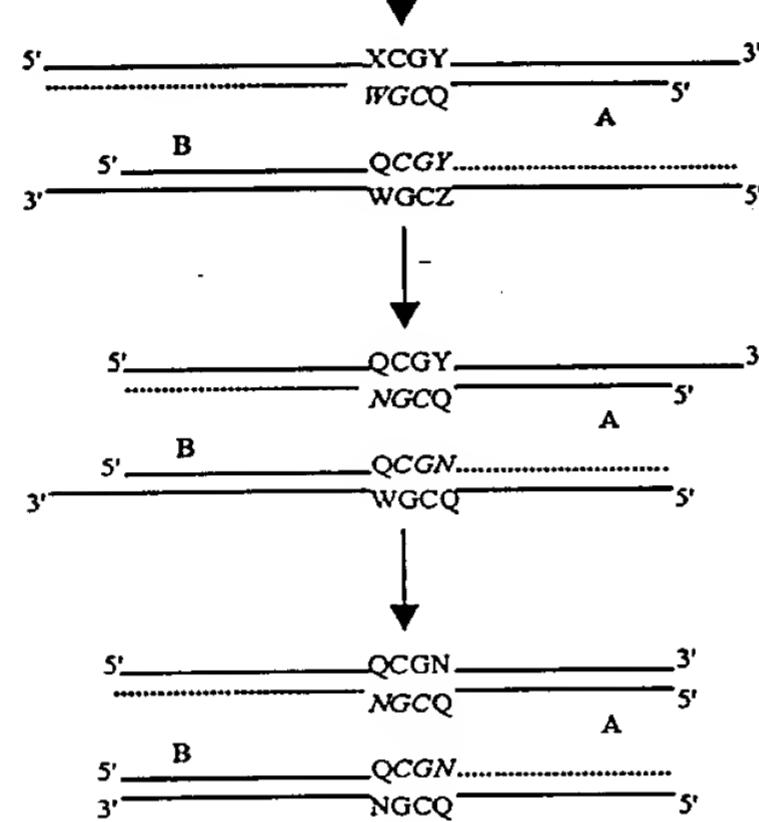
WILD-TYPE

MUTANT

1)



2)



3)

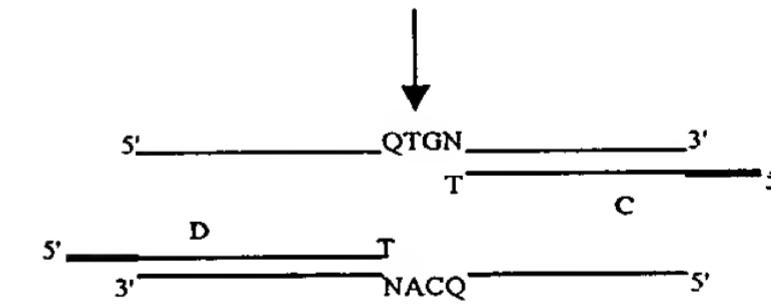
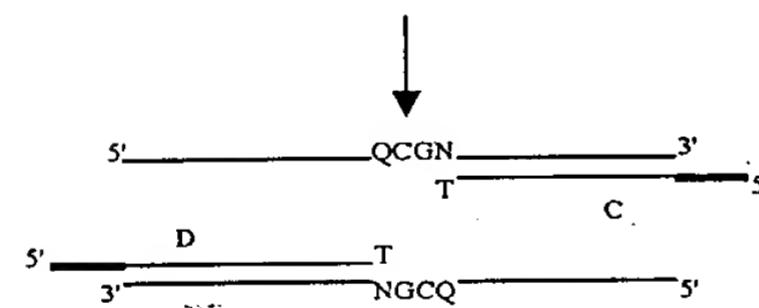
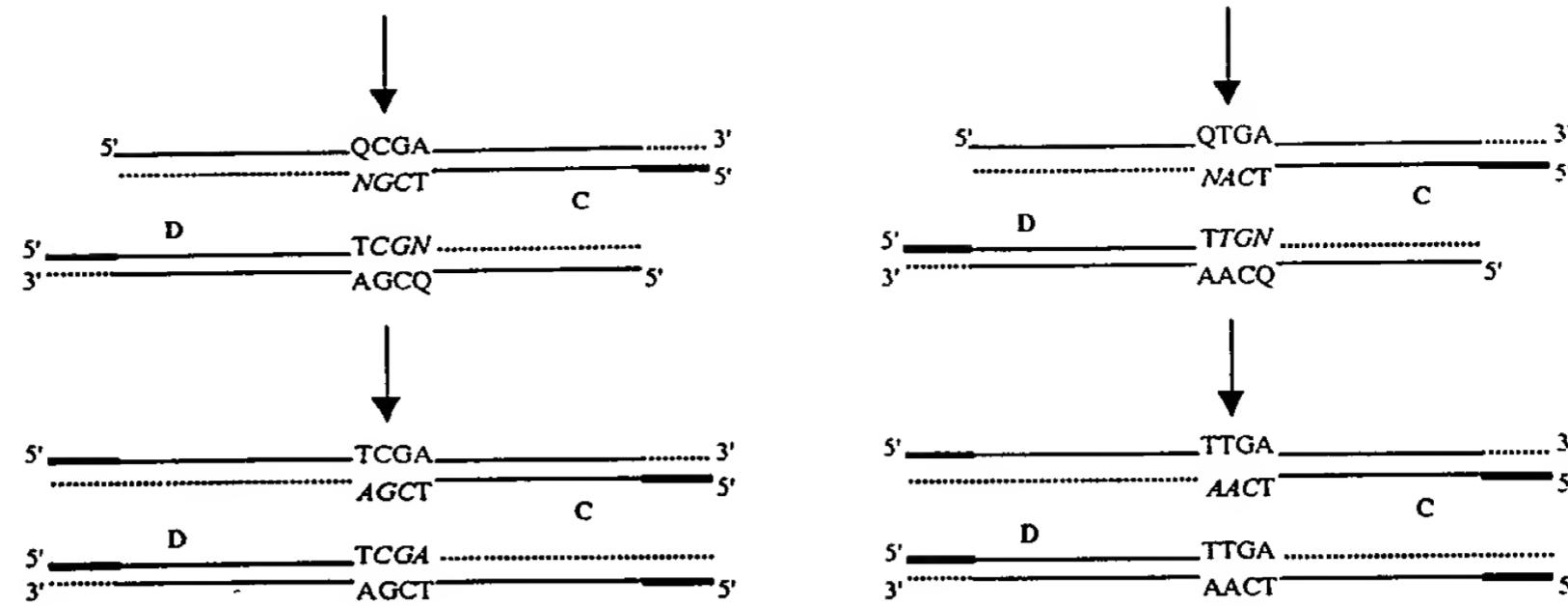


FIGURE 1

4)



5)

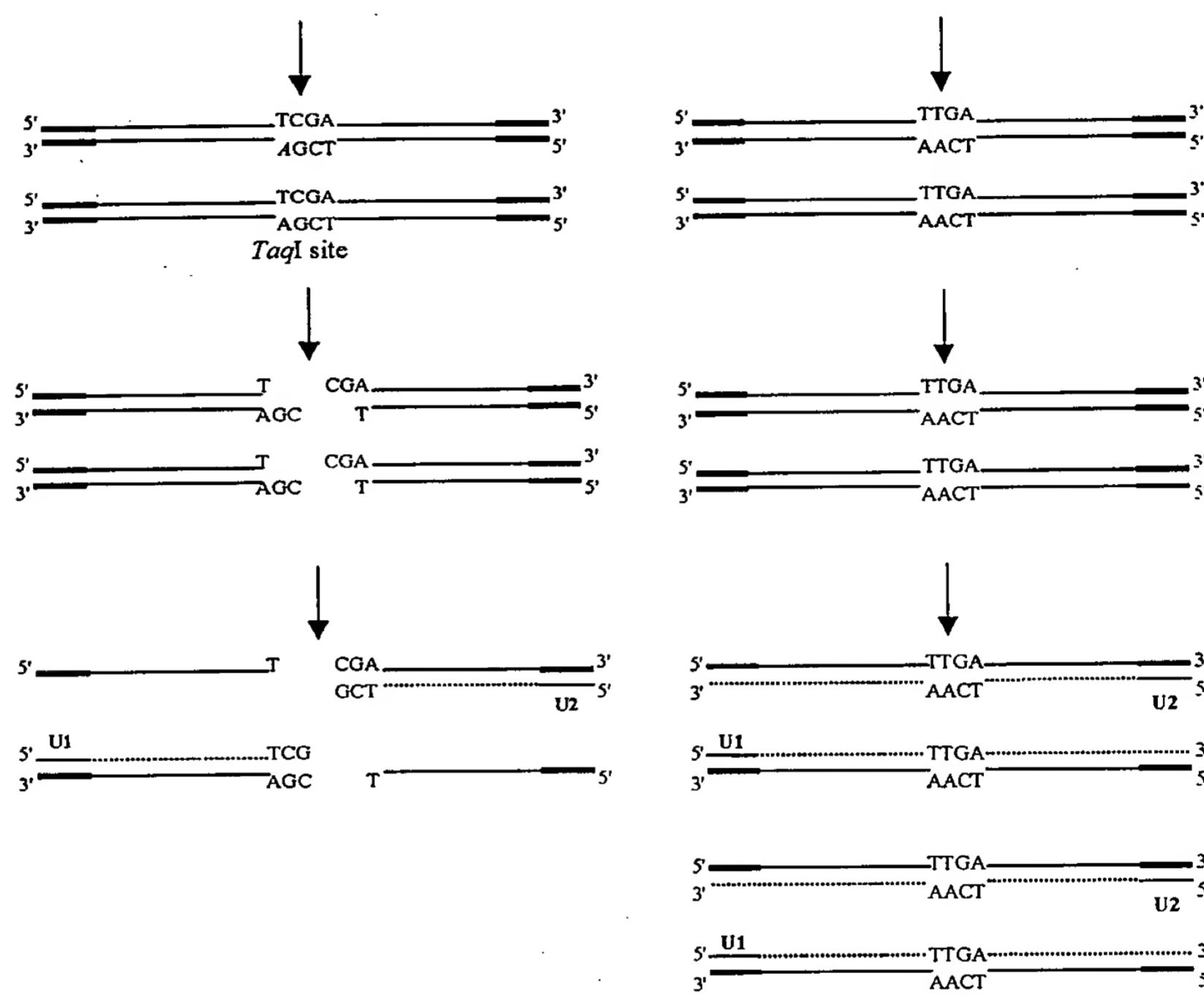
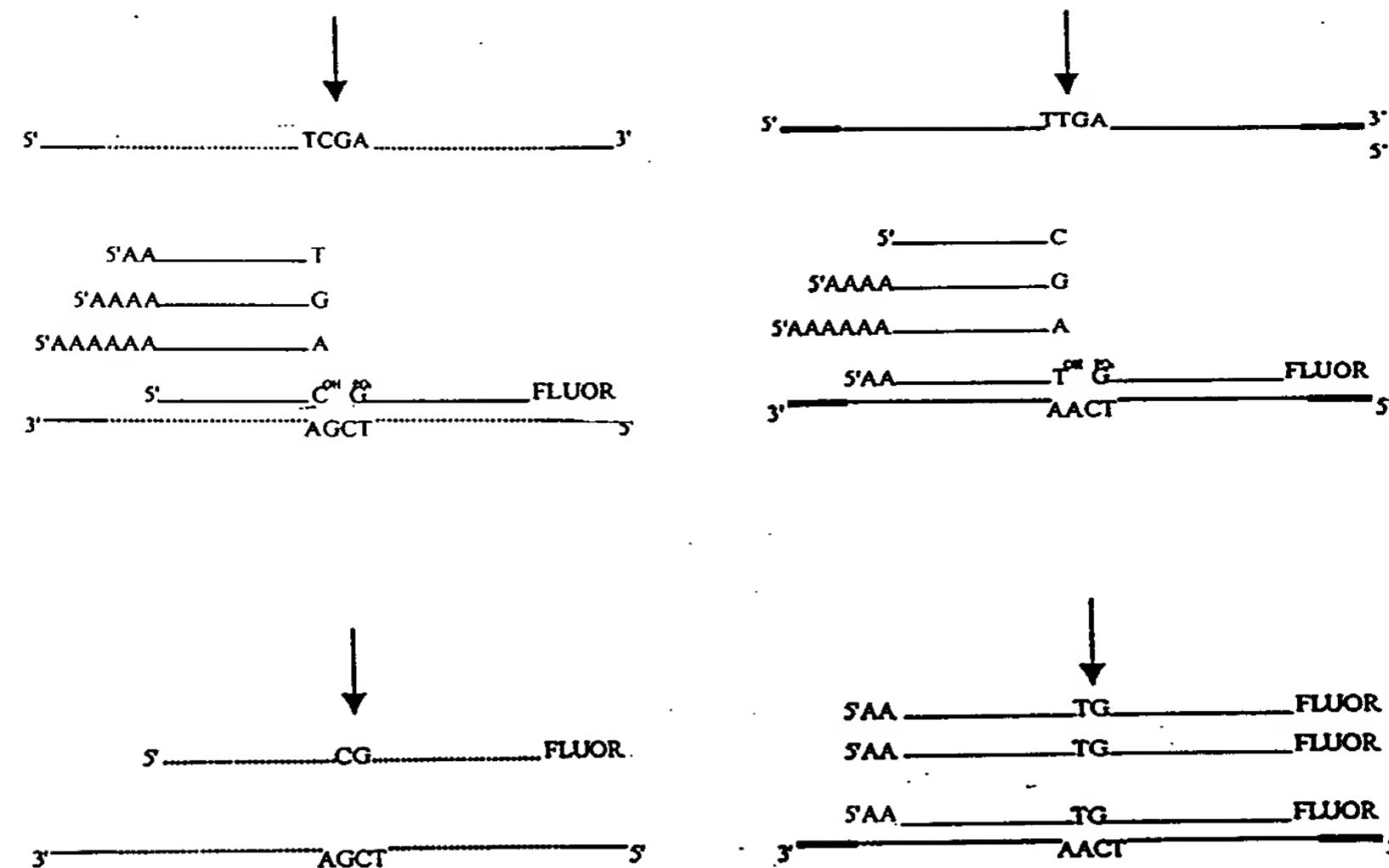


FIGURE 1 (Cont'd.)

7A)



7B)

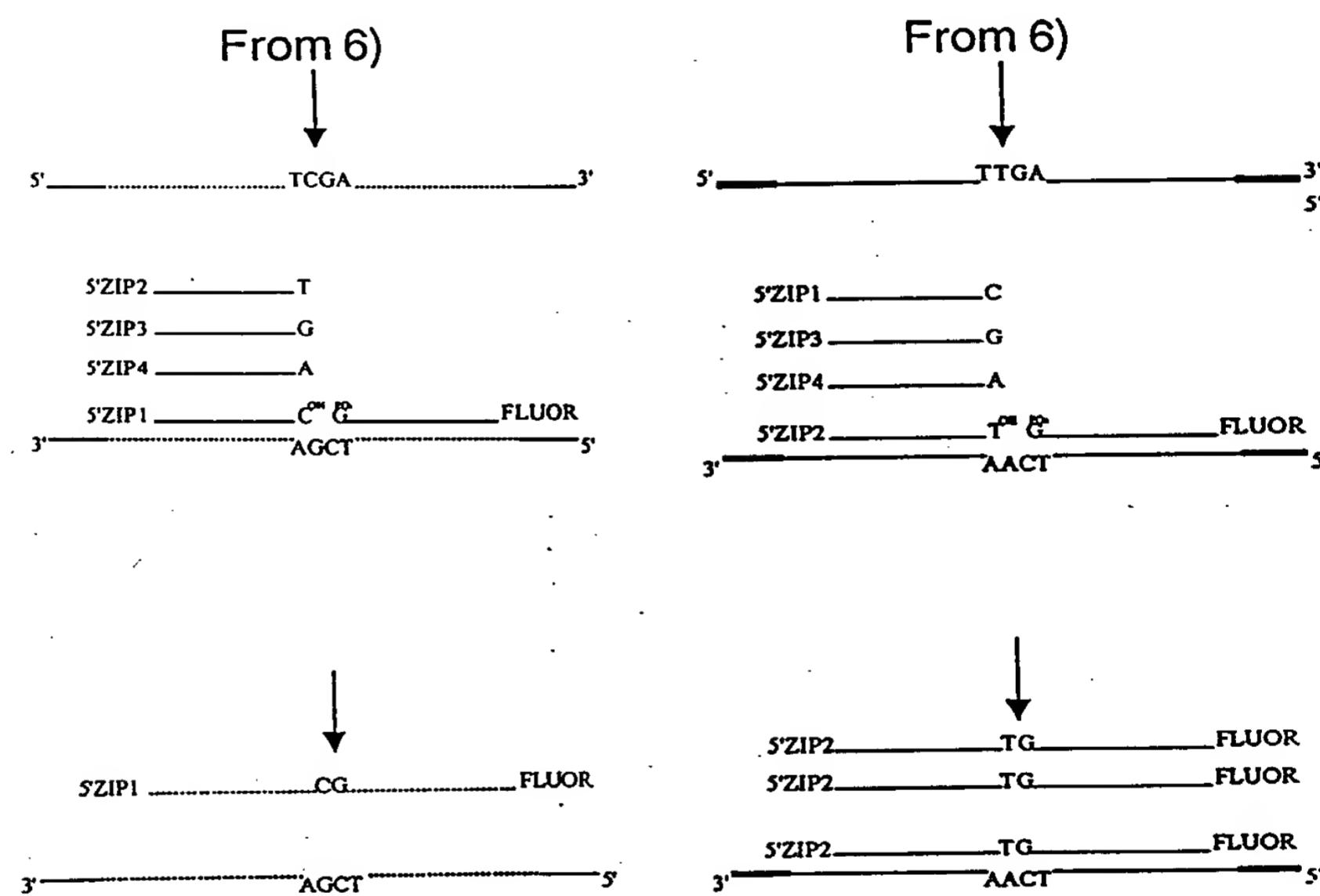


FIGURE 1 (Cont'd.)

I. GEL LANE

S'AA TG FL

S' CG FL

S'AAAAAA A

S'AAA G

S'G FL

S'AA T

S' C

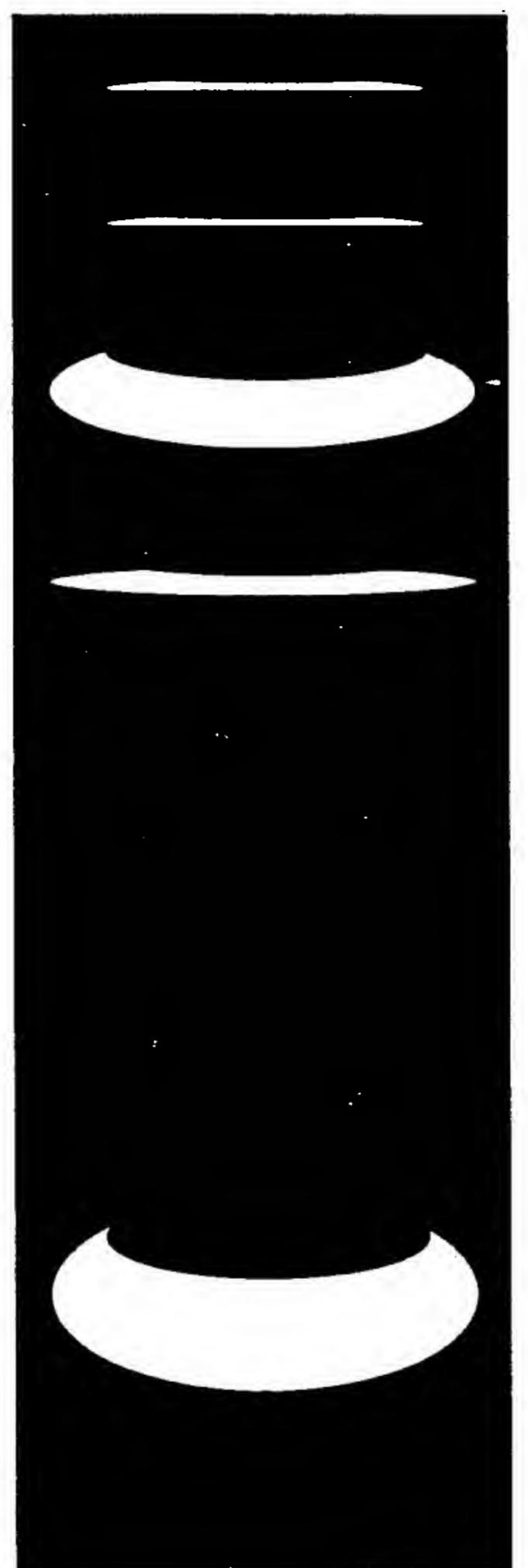


FIGURE 2

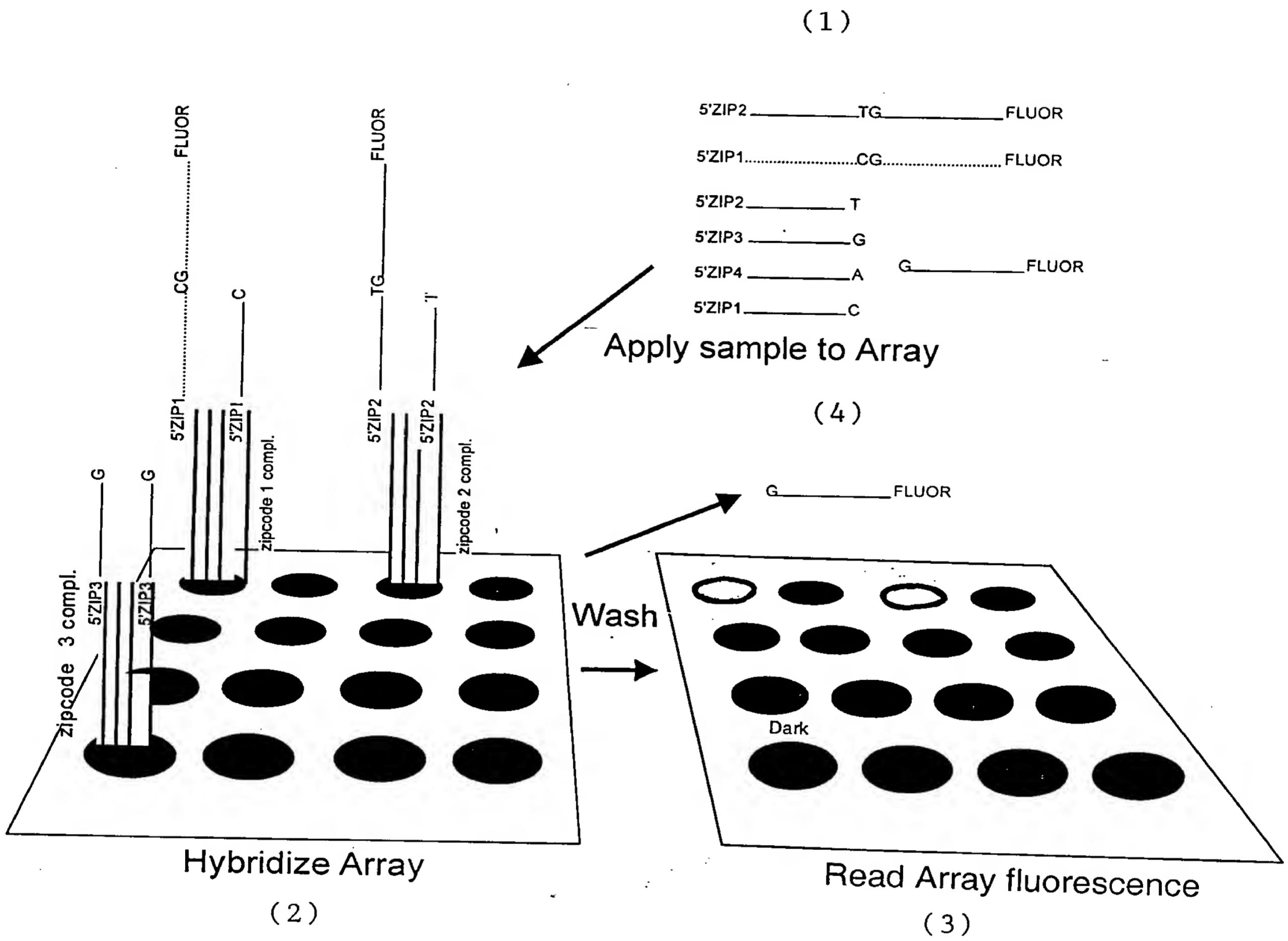


FIGURE 3

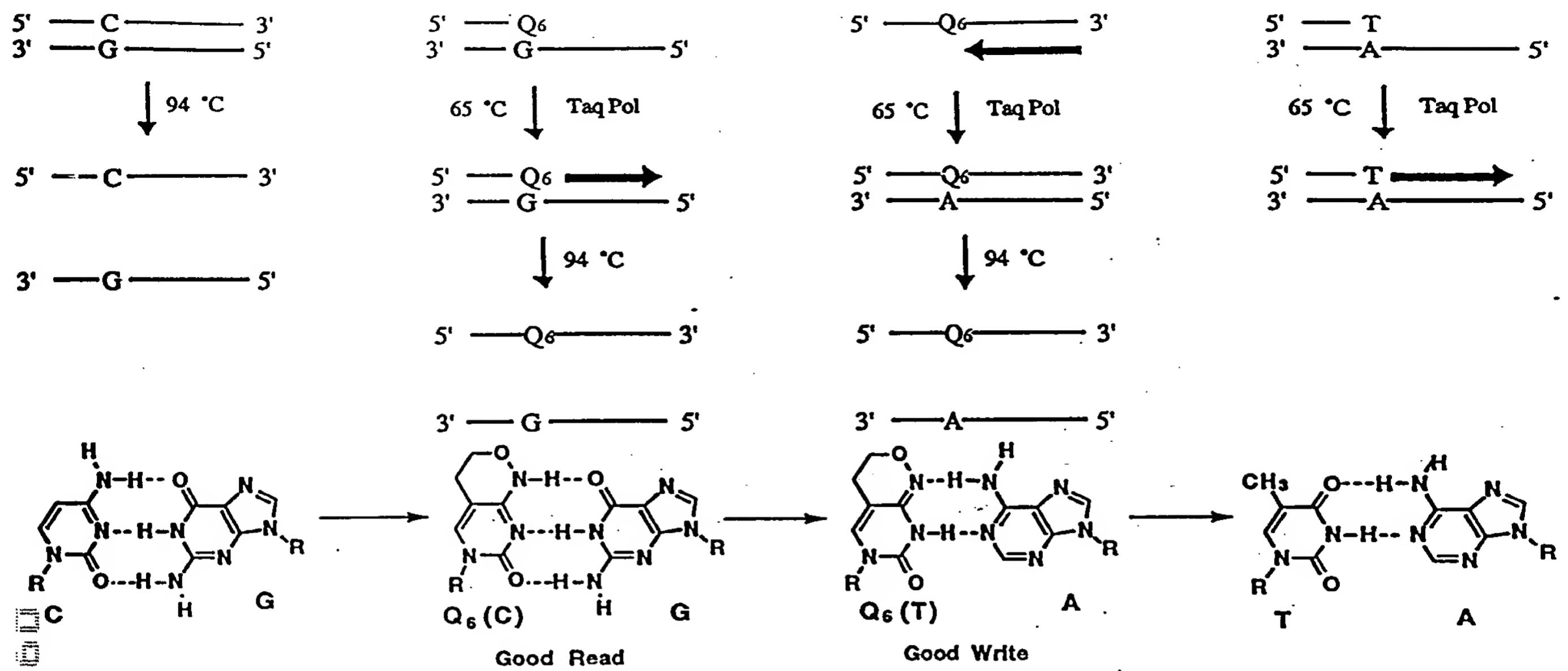


FIGURE 4

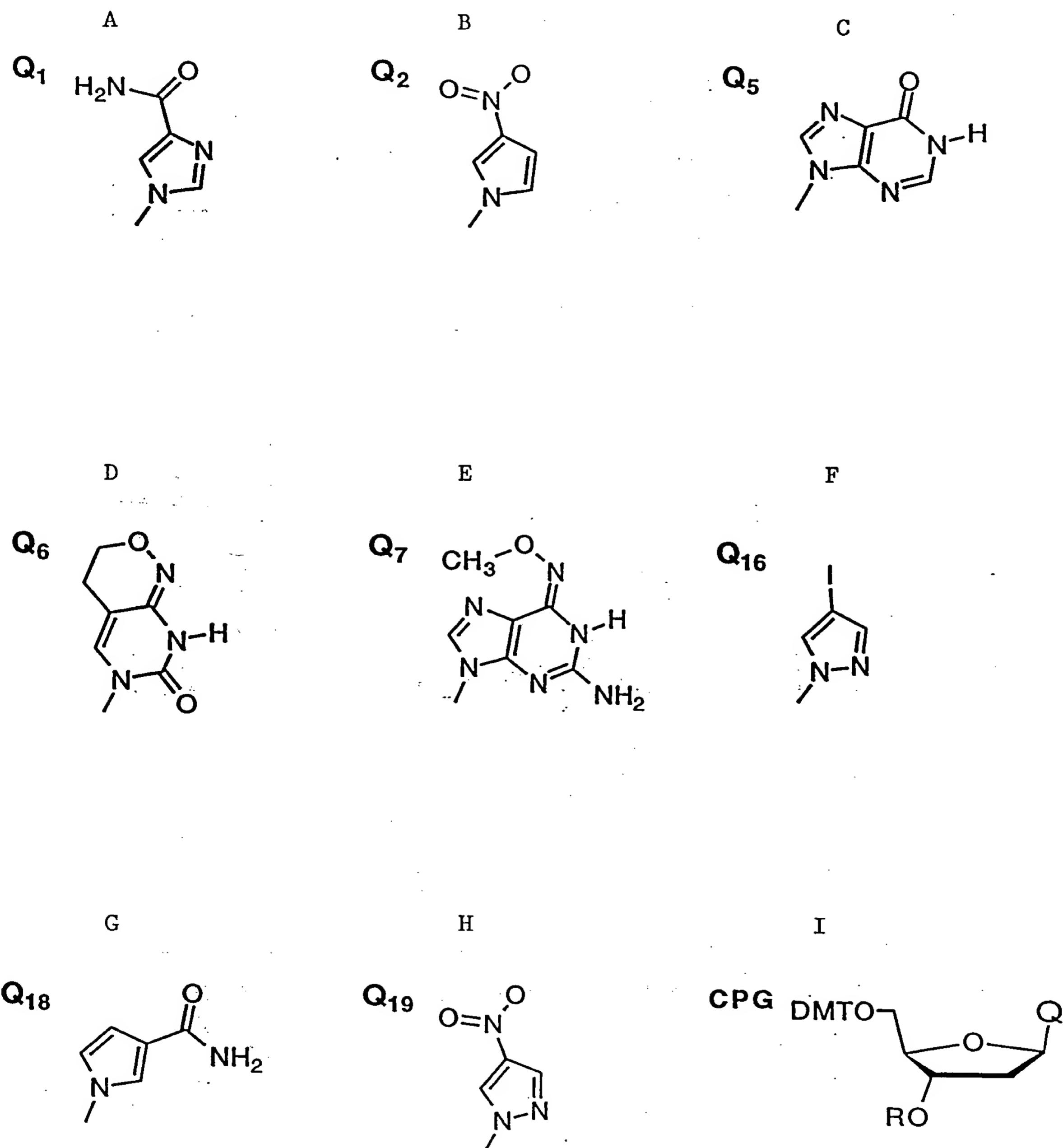


FIGURE 5

A

Primers

Ztop

CTT CGA CGA GTT CAT ACG C

codon 248

↓

p53zip248

CTT CGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG A

p53-248X

T TCT TCC TGC ATG GGC GGC ATG AAX → pol

|| ||| ||| ||| ||| ||| ||| |||

50 bp synthetic
duplex DNA

3' CA AGG AGC TAC CGG CGG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TGC ATG GGC GGC ATG AAC CGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| |||

pol-X TCC CGG TAG GAG TGG TAG TAG TGT T

C GGG TAG GAG TGG TAG TAG TGC ACC GCT CGG TCA AAC G

C ACC GCT CGG TCA AAC G

p53-248XR

p53zip248R

Zbot

B

Primers

Ztop

CTT CGA CGA GTT CAT ACG C

codon 248

↓

p53zip248T

CTT CGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG AAT

p53-248QN

T TCT TCC TGC ATG GGC GGC ATG AACN → pol

|| ||| ||| ||| ||| ||| ||| |||

50 bp synthetic
duplex DNA

3' CA AGG AGC TAC CGG CGG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TGC ATG GGC GGC ATG AAC CGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| |||

pol-QN TCC CGG TAG GAG TGG TAG TAG TGT T

T TCC CGG TAG GAG TGG TAG TAG TGC ACC GCT CGG TCA AAC G

C ACC GCT CGG TCA AAC G

Zbot

C

LDR Primers

p53LDR248FCA

Discrimination

Common

F-AAAAAAA GC ATG GGC GGC ATG AAC A

F-AAAAA GC ATG GGC GGC ATG AAC G

F-AA GC ATG GGC GGC ATG AAC T

F- GC ATG GGC GGC ATG AAC C

7-ligase

p53LDR248FCG

GG AGG CCC ATC CTC ACC ATC AT-block

p53LDR248FCT

3' (-strand) ... GTC GCA AGG ACG TAC CGG CGG TAC TTG NCC TCC GGG TAG GAG TGG TAG TAG TGA ACC ... 5'

p53LDR248FCC

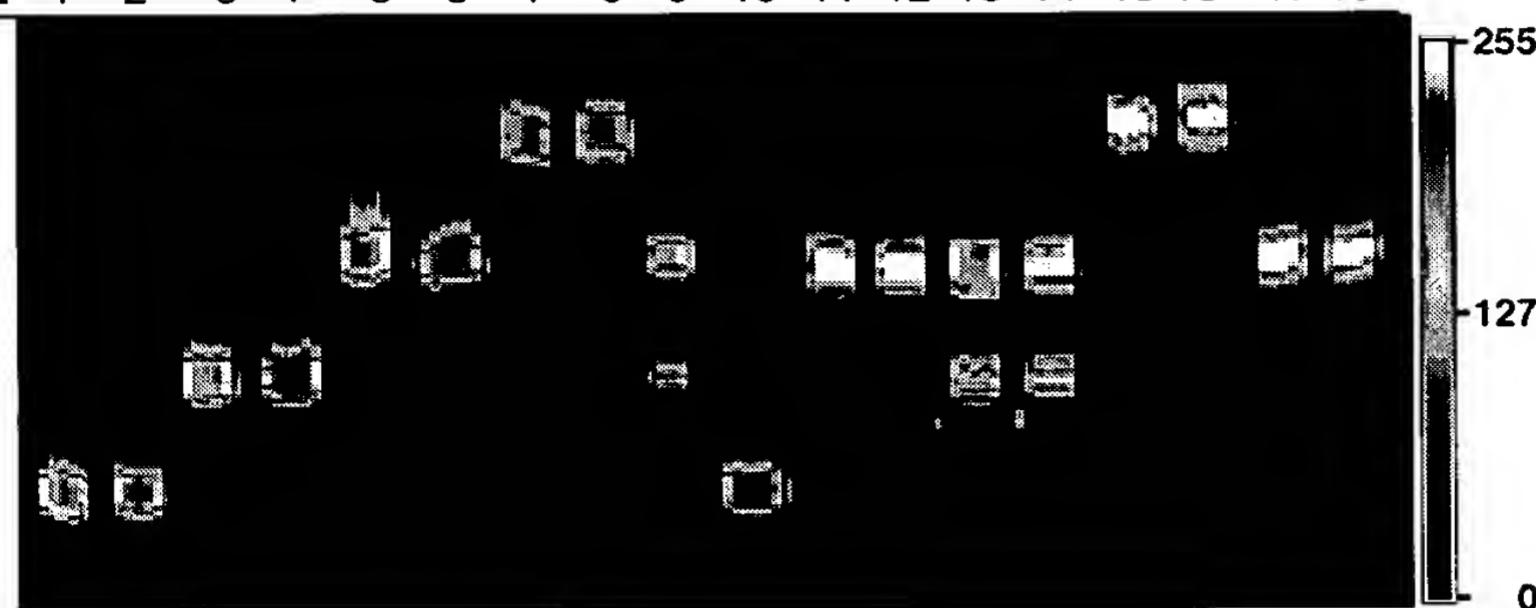
conversion

products

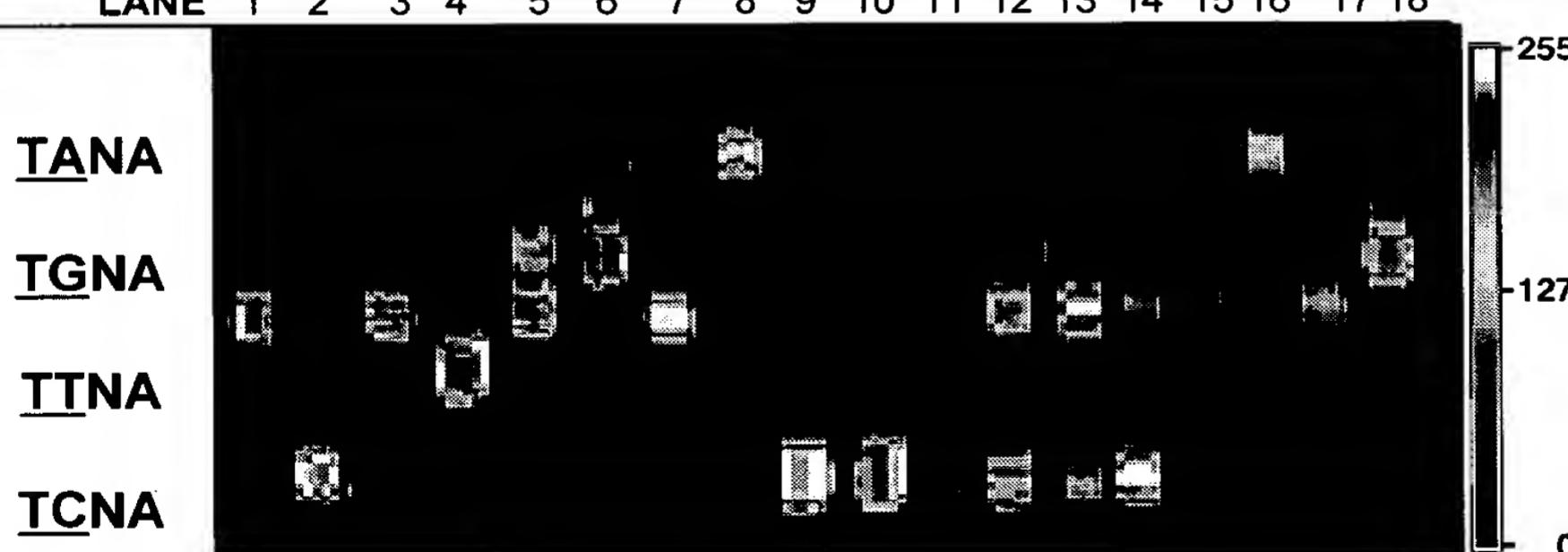
FIGURE 6

A

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG										
Expctd prod	<u>CCGG</u>	<u>CTGG</u>	<u>CGGG</u>	<u>CAGG</u>	<u>CCGG</u>	<u>CCGG</u>	<u>CATG</u>	<u>CGCG</u>											
primer 3' end	C	Q ₆	C																
1st base	C	C	C	C	C	C	C	C	C										
2nd base	C	C	T	T	G	A	A	G	G										
minor 2nd base prod					t	t	t	T	T										
	LANE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

**B**

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG										
Expctd prod	<u>TCGA</u>	<u>TTGA</u>	<u>TGGA</u>	<u>TAGA</u>	<u>TCGA</u>	<u>TCGA</u>	<u>TCGA</u>	<u>TATA</u>	<u>TGCA</u>										
primer 3' end	T	Q ₆	T																
1st base	T	T	T	T	T	T	T	T	T										
2nd base	?	C	?	T	?	G	?	A	?										
minor 2nd base prod					g		?	c	?										
	LANE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

**FIGURE 7**

A

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	<u>GC</u> GC	<u>GT</u> GC	<u>GG</u> GC	<u>GAG</u> C	<u>GCG</u> C	<u>GCG</u> C	<u>GCG</u> C	<u>GAT</u> C	<u>GG</u> CC
primer 3' end	G Q ₅ Q ₇								
1st base	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	?
minor 2nd base prod		t t t	g g	A A a					

LANE 1 2 4 5 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
3 6

**B**

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	<u>AC</u> GT	<u>AT</u> GT	<u>AG</u> GT	<u>AAG</u> T	<u>AC</u> GT	<u>AC</u> GT	<u>AC</u> GT	<u>AAT</u> T	<u>AG</u> CT
primer 3' end	A Q ₇ Q ₅								
1st base	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	c C c
minor 2nd base prod	?	?							

LANE 1 2 4 5 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
3 6



FIGURE 8

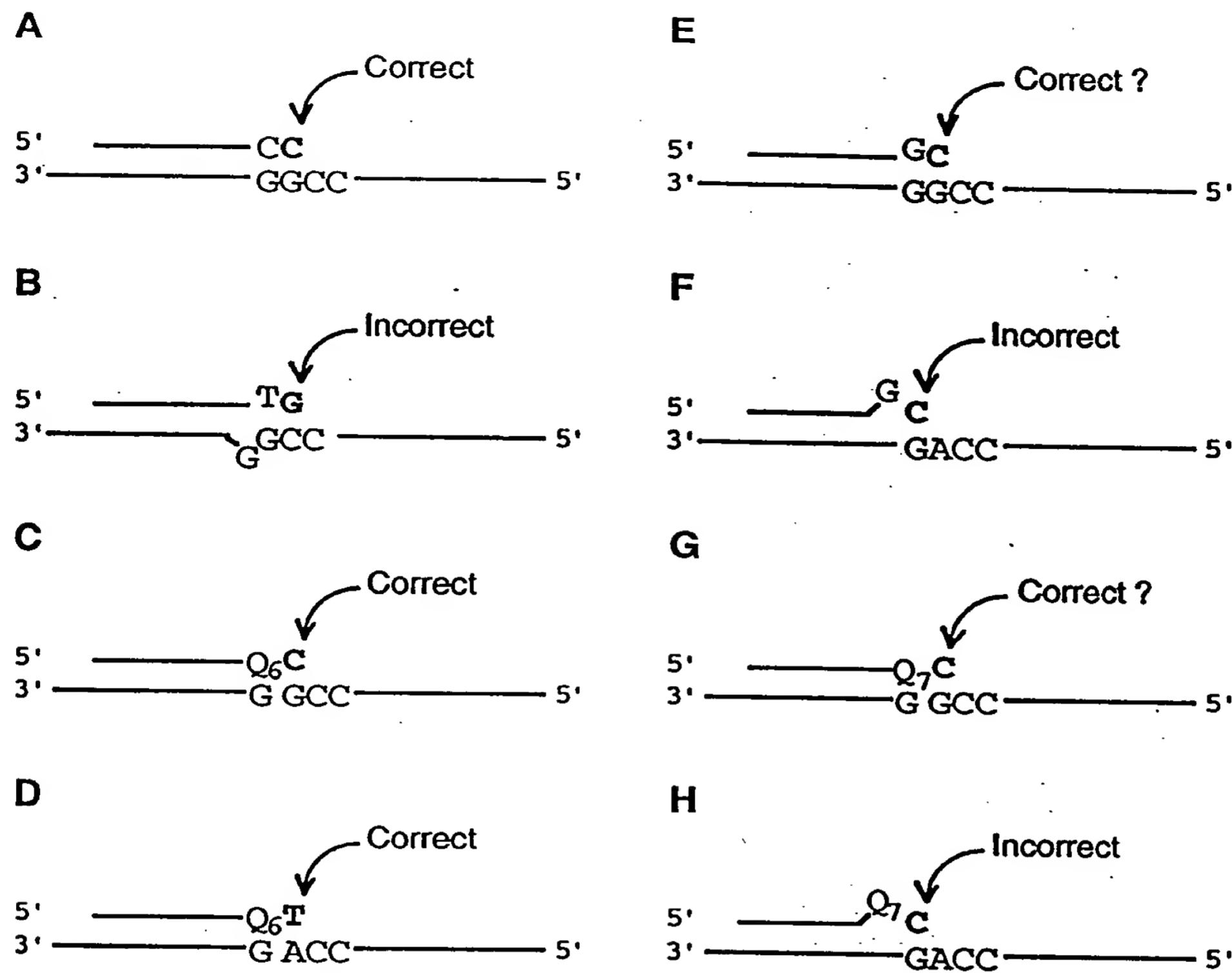
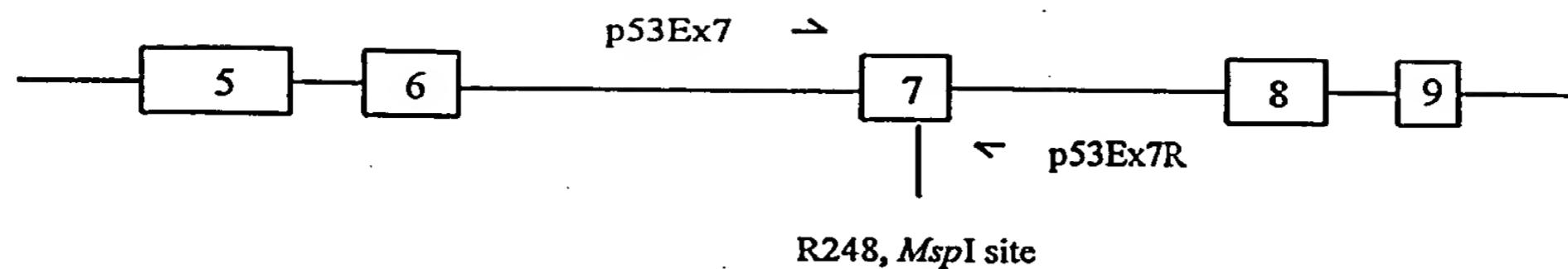
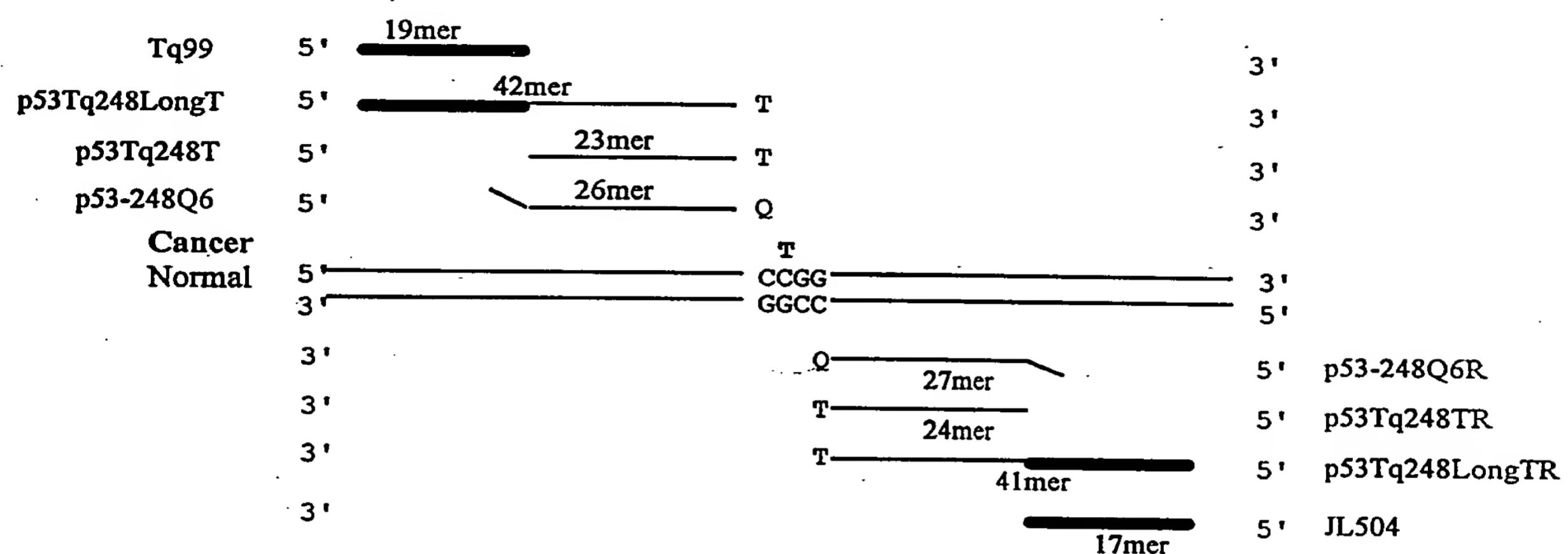
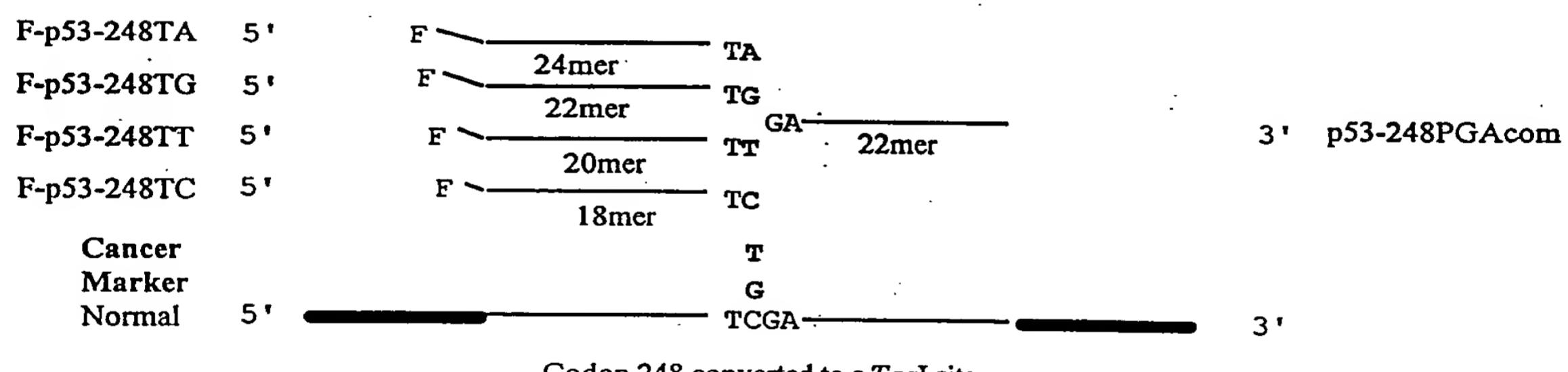


FIGURE 9

A**B**Cancer
Normal**FIGURE 10**

A

Primers

Ztop CTT GGA CGA GTT CAT ACG C
 ↓
 p53zip248short CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GCC GGC ATG A
 p53-248short GT TCC TGC ATG GCC GGC A → pol
 ||| ||| ||| ||| ||| ||| ||| |||
 p53 exon 7 3' ... CA AGG AGC TAC CCG CCG TAC TTC GCC TCC CGG TAG GAG TGG TAG TAG TGT ... 5' (-)
 PCR product (MK not shown)
 p53-248shortR 5' ... GT TCC TGC ATG GCC GGC ATG AAC GCG AGG CCC ATC CTC ACC ATC ATC ACA ... 3' (+)
 ||| ||| ||| ||| ||| ||| ||| |||
 pol-GG TAG GAG TGG TAG TAG TG
 C CGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC C
 p53zip248shortR C ACC GCT CGG TCA AAC C
 Zbot

B

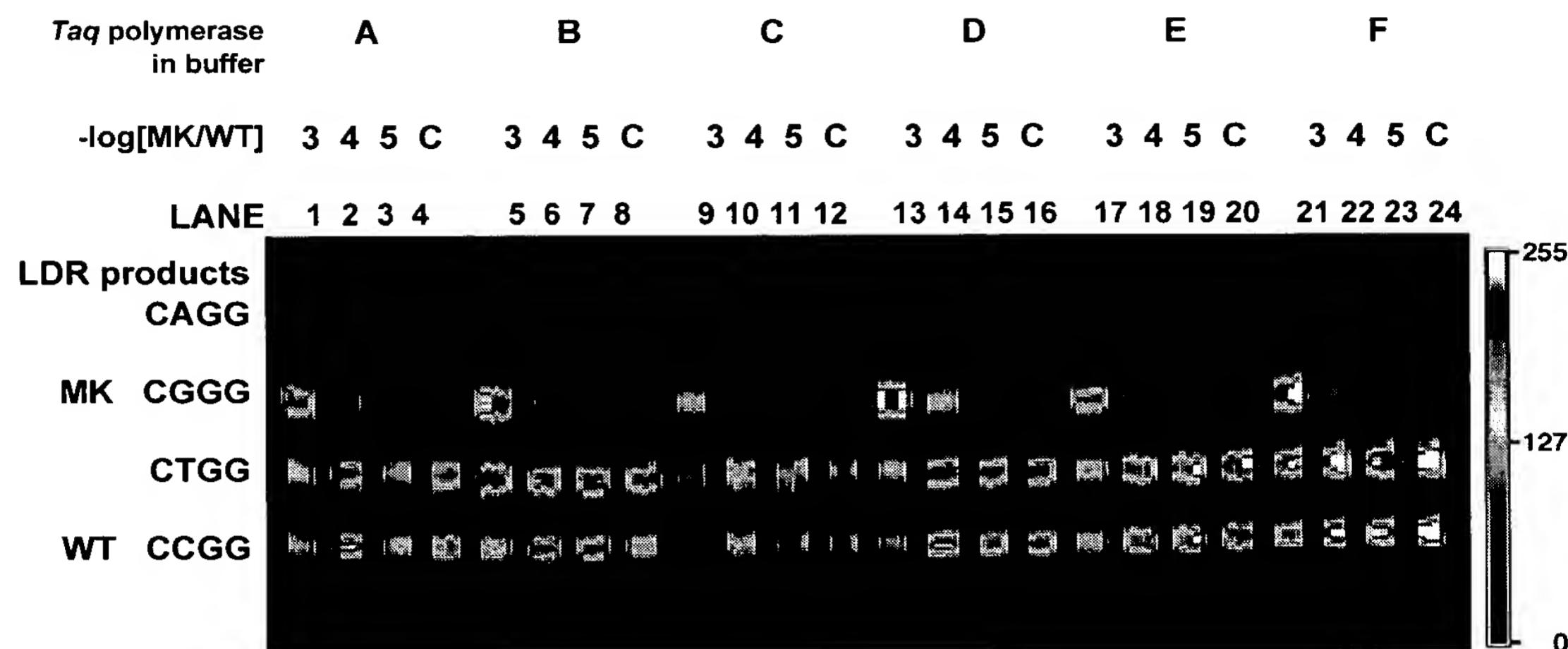
Primers

LDR Primers

p53LDR248FTCL F-AAAAAAA GC ATG GGC GGC ATG AAT C
 p53LDR248FCA F-AAAAAA GC ATG GGC GGC ATG AAC A
 p53LDR248FCG F-AAAA GC ATG GGC GGC ATG AAC G
 p53LDR248FCT F-AA GC ATG GGC GGC ATG AAC T
 p53LDR248FCC F- GC ATG GGC GGC ATG AAC C γ -ligase
 p53LDR248PGG GG AGG CCC ATC CTC ACC ATC AT-block
 conversion 3' (-strand) || ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
 products ... GTA TGC GCA AGG ACG TAC CCG CGG TAC TTG NGG TCC CGG TAG GAG TGG TAG TAG TGA ACC...

FIGURE 11

A



B

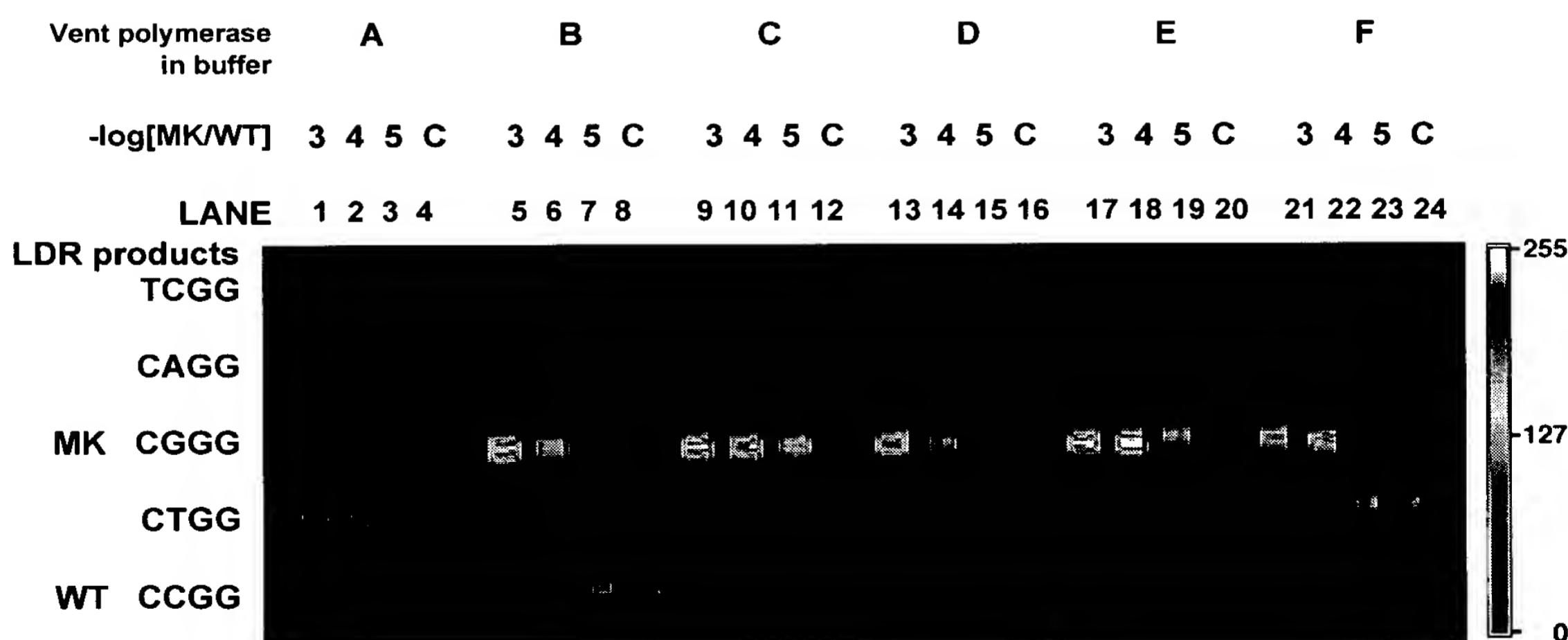


FIGURE 12

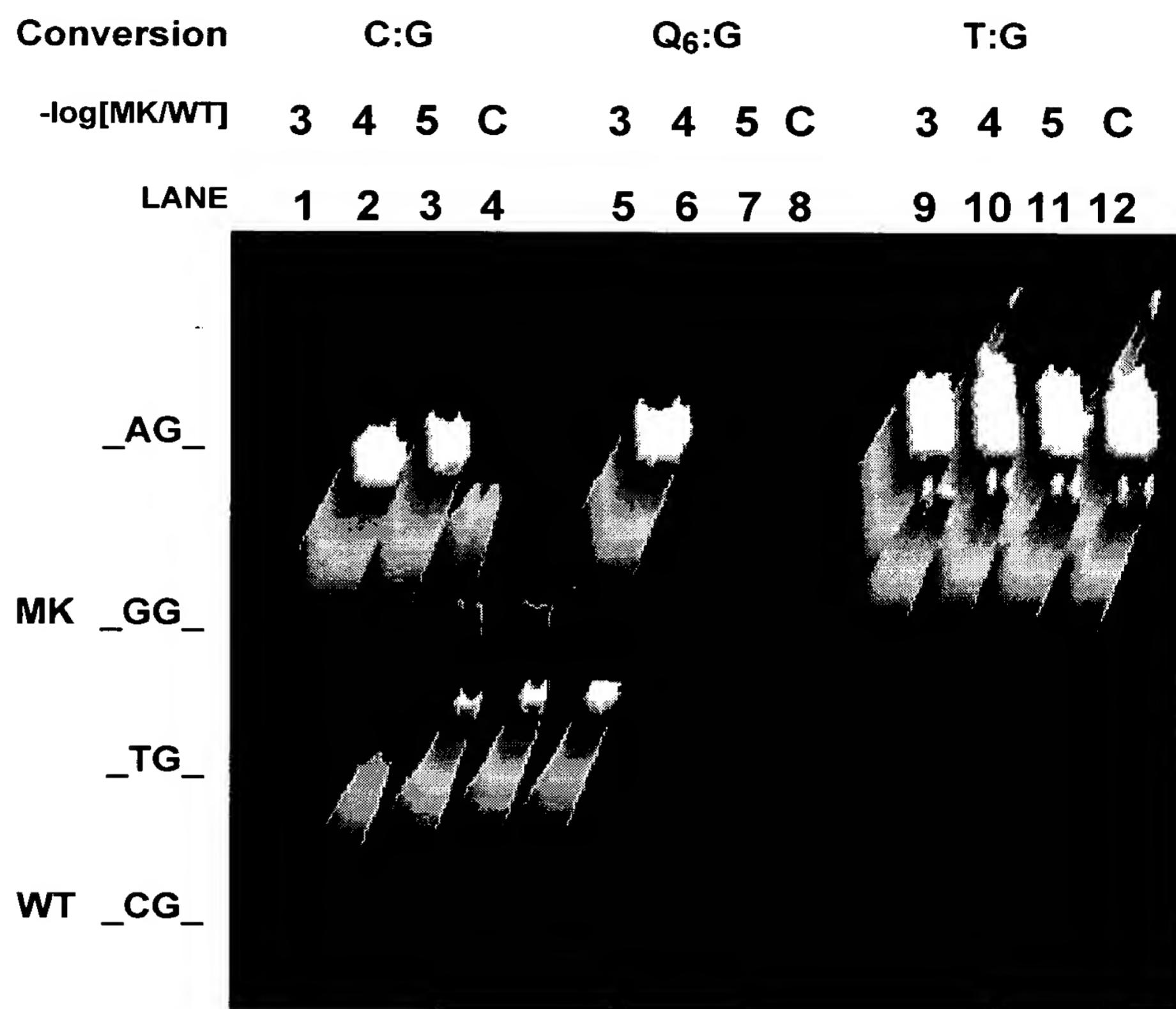


FIGURE 13